PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2003-235400
(43)Date of publication of application : 26.08.2003

(51)Int.Cl. A01K 67/027
A61K 45/00
A61P 13/12
A61P 43/00
C12N 15/09
G01N 33/15
G01N 33/50
G01N 33/566

(21)Application number : 2002-033148
(22)Date of filing : 08.02.2002

(71)Applicant : MIYATA TOSHIO
JAPAN SCIENCE & TECHNOLOGY CORP
(72)Inventor : MIYATA TOSHIO

(54) MODEL ANIMAL WITH LIVER FUNCTION FAILURE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a model animal with liver function failure, having failure in repair function of failure in glomerulus.
SOLUTION: The method for producing the model animal with the liver function failure, having the failure in the repair function in the glomerulus involves a step for damaging the glomerulus of an animal having enhanced expression of a megsin gene in the glomerulus. The model animal with the liver function failure is new. The model animal is useful for the research of

the repair mechanism of the glomerulus and the screening of a therapeutic agent of human renal dysfunction.

LEGAL STATUS

[Date of request for examination] 23.07.2004

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

CLAIMS

[Claim(s)]
[Claim 1] The manufacture approach of a renal dysfunction model animal that the restoration function including the process which carries out the failure of the glomerulus of the animal which reinforced the Meg Singh gene expression in a glomerulus of the breakage in a glomerulus was checked.
[Claim 2] The approach according to claim 1 of carrying out the failure of the glomerulus by the antibody to a glomerulus organization.
[Claim 3] The approach according to claim 2 an antibody is an antibody combined with glomerular capillary basement membrane.
[Claim 4] The approach according to claim 3 an antibody is the antibody with which said animal was medicated, or the autoantibody guided in said animal according to the immunity of a glomerular capillary basement membrane antigen.
[Claim 5] The renal dysfunction model animal from which the restoration function of the breakage in a glomerulus to have the following description (a) and (b) was prevented.
(a) The hemodialysis function and the gestalt-description of the (b) glomerulus which the Meg Singh gene expression in a glomerulus is reinforcing are normal [Claim 6] substantially. How to measure the activity which reinforces the restoration function including the following process of the failure in the glomerulus of a test compound.
(1) The process which prepares the renal dysfunction model animal from which the restoration function of the breakage in a glomerulus to have the following description (a) and (b) was prevented, (a) -- the hemodialysis function and the gestalt-description of the (b) glomerulus which the Meg Singh gene expression in a glomerulus is reinforcing -- substantial -- normal (2) -- the process which does breakage to the glomerulus of said animal -- (3) The process which medicates said animal with a test compound in front of a process (2) and/or in the back, and process which evaluates extent of

the recovery of damage in (4) glomeruli [claim 7] The screening approach of a compound of having the activity which reinforces the restoration function of the breakage in a glomerulus which measures the activity which reinforces the restoration function of the failure in the glomerulus of a test compound by the approach according to claim 6, and includes the process as which said activity chooses a large compound as compared with the contrast which does not prescribe a test compound for the patient.
[Claim 8] Drugs for restoring breakage on a glomerulus which contain the compound chosen by the approach according to claim 7 as an active principle.
[Claim 9] How to carry out the failure of the function which restores the breakage including the process which reinforces equivalent gene expression on Meg Singh or Meg Singh, and a functional target in a kidney organization in the glomerulus of a nonhuman vertebrate.
[Claim 10] The nonhuman vertebrate to which the failure of the function which restores the breakage in a glomerulus which consists of an animal which reinforced equivalent gene expression on Meg Singh or Meg Singh, and a functional target in the kidney organization was carried out.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a renal dysfunction model animal.
[0002]
[Description of the Prior Art] The model animal of renal dysfunction is useful in a break through of the failure device of a kidney function, and development of the remedy of renal dysfunction. Many renal dysfunction model animals are well-known. The well-known nephritis model animal was obtained by carrying out the failure of the kidney function artificially. It considers as a renal dysfunction model, for example, the following model animals are well-known.
[0003] GBM [anti-] nephritis: If an animal is medicated with the antibody which recognizes a glomerular capillary basement membrane (GBM) antigen, it is well-known that the renal dysfunction model accompanied by symptoms, such as are recording of the immune complex to a glomerulus, is establishable. In a GBM nephritis model animal, the failure of the kidney function is carried out by administration of an anti-GBM antibody transient. However, since there is no problem in a repair mechanism, renal dysfunction is recovered soon.
[0004] Meg Singh transgenic animal: this invention persons isolated Meg Singh as a gene specifically discovered to a mesangial cell. And the transgenic animal which introduced Meg Singh showed clearly that it is useful as a model animal of a mesangial nephritis. In the mesangium organization of the Meg Singh TRANS GENIC mouse which greeted about 35 to 40 weeks old, the deposition of complement and the immune complex which consists of an immunoglobulin is accepted in the hyperplasia of the Tsuguaki cell proliferation which makes a mesangial cell a subject, and a mesangium substrate, and a list, and having lapsed into hardening (segmental sclerosis) of metamerism is observed. However, in this model animal, it is not known

that abnormalities will be looked at by the repair mechanism of the kidney function by which the failure was carried out.

[0005]

[Problem(s) to be Solved by the Invention] The technical problem of this invention is offer of the model animal which has a failure in the repair mechanism of the breakage in a glomerulus. Moreover, this invention offers a technical problem the screening approach of a compound useful for the therapy of the disease resulting from the failure of the repair mechanism of the breakage in a glomerulus.

[0006]

[Means for Solving the Problem] It is already shown clearly by this invention person that Meg Singh's compulsive manifestation in a glomerulus causes a mesangial nephritis. this invention person advanced research further about the cause of the renal dysfunction by Meg Singh, and found out that restoration of the breakage in a glomerulus was overdue with Meg Singh. And the animal which reinforced Meg Singh's manifestation in a glomerulus showed clearly that it is useful as a model animal which has a failure in restoration of the breakage in a glomerulus, and completed this invention.

[0007] That is, this invention relates to the following renal dysfunction model animal, its manufacture approach, and the screening approach that used this model animal for the list.

[1] The manufacture approach of a renal dysfunction model animal that the restoration function including the process which carries out the failure of the glomerulus of the animal which reinforced the Meg Singh gene expression in a glomerulus of the breakage in a glomerulus was checked.

[2] An approach given in [1] which carries out the failure of the glomerulus by the antibody to a glomerulus organization.

[3] An approach given in [2] whose an antibody is an antibody combined with glomerular capillary basement membrane.

[4] An approach given in [3] whose an antibody is the antibody with which said animal was medicated, or the autoantibody guided in said animal according to the immunity of a glomerular capillary basement membrane antigen.

[5] The renal dysfunction model animal from which the restoration function of the breakage in a glomerulus to have the following description (a) and (b) was prevented.

(a) the hemodialysis function and the gestalt-description of the (b) glomerulus which the Meg Singh gene expression in a glomerulus is reinforcing -- substantial -- normal [6] -- the approach of measuring the activity which reinforces the restoration function including the following process of the failure in the glomerulus of a test compound.

(1) The process which prepares the renal dysfunction model animal from

which the restoration function of the breakage in a glomerulus to have the following description (a) and (b) was prevented, (a) -- the hemodialysis function and the gestalt-description of the (b) glomerulus which the Meg Singh gene expression in a glomerulus is reinforcing -- substantial -- normal (2) -- the process which does breakage to the glomerulus of said animal -- (3) The process which medicates said animal with a test compound in front of a process (2) and/or in the back, And measure the activity which reinforces the restoration function of the failure in the glomerulus of a test compound to the process [7] and [6] which evaluate extent of the recovery of damage in (4) glomeruli by the approach of a publication, and it compares with the contrast which does not prescribe a test compound for the patient. The screening approach of a compound of having the activity which reinforces the restoration function including the process which chooses a compound with said large activity of the breakage in a glomerulus.
Drugs for restoring breakage on a glomerulus which contain the compound chosen as [8] and [7] by the approach of a publication as an active principle.
[9] How to carry out the failure of the function which restores the breakage including the process which reinforces equivalent gene expression on Meg Singh or Meg Singh, and a functional target in a kidney organization in the glomerulus of a nonhuman vertebrate.
[10] The nonhuman vertebrate to which the failure of the function which restores the breakage in a glomerulus which consists of an animal which reinforced equivalent gene expression on Meg Singh or Meg Singh, and a functional target in the kidney organization was carried out.
[0008]
[Embodiment of the Invention] In this invention, the failure of the repair mechanism of the breakage in a glomerulus means that the function which restores the failure after removing the cause of breakage falls. For example, when a normal mouse is medicated with an anti-GMB antibody and an anti-GBM nephritis is started, renal dysfunction is transient and a kidney function is recovered soon. In the transgenic animal which, on the other hand, carried out the forcible manifestation of Meg Singh in the kidney organization, in order that restoration of a glomerulus function may be overdue, restoration of the renal dysfunction after antibody administration is remarkably overdue. In this invention, not only when recovery is not expected for a failure to be unrestorable, but the case where recovery is overdue compared with a wild type is included.
[0009] Moreover, in this invention, the failure of a repair mechanism includes the case where it is in the condition of not doing breakage. That is, if the repair mechanism is imperfect when breakage is done, it will not be concerned with the existence of breakage but it will be said that it is in the condition that the failure of the repair mechanism was carried out. Therefore,

the renal dysfunction model animal of this invention contains the animal in the condition that breakage is not done. Moreover, renal dysfunction means the condition that the hemodialysis function by the glomerulus fell. The breakage on the glomerulus in this invention means breakage on the arbitration which bars the function of a glomerulus. For example, the immunological breakage by the antibody which recognizes the organization which constitutes a glomerulus is mentioned.

[0010] This invention relates to the manufacture approach of a renal dysfunction model animal that the restoration function including the process which carries out the failure of the glomerulus of the animal which reinforced the Meg Singh gene expression in a glomerulus of the breakage in a glomerulus was checked. The animal which reinforced the Meg Singh gene expression in the glomerulus used for this invention can be obtained by introducing the Meg Singh gene and carrying out a forcible manifestation in a mesangial cell. Or it can act on the promotor of the Meg Singh gene, and the Meg Singh gene expression can also be reinforced by administration of the compound which promotes the Meg Singh gene expression. The acquisition approach of the drugs which act on the promotor of the Meg Singh gene and this promotor is well-known (WO 00/43528).

[0011] The animal which reinforced Meg Singh's manifestation in the mesangial cell used for this invention can be obtained by the production approach of a well-known transgenic animal. A transgenic animal is produced according to a "developmental engineering experiment manual" (the volume Tatsuji Nomura editorial supervisions and for Motonari Katsuki, Kodansha, 1989), "a new chemistry experiment lecture, an animal experiment method, etc." (the edited by Japanese Biochemical Society, Tokyo Kagaku Dojin, 1991), etc. Below, it states according to the production protocol of a common transgenic animal. In addition, the protein in which a code is carried out to the Meg Singh gene and a list by this gene is well-known (WO 99/15652). Furthermore, the transgenic animal which this invention person made carry out the forcible manifestation of this DNA in a mesangial cell showed clearly that it is useful as a model animal of a mesangial cell fecundity nephritis (WO 01/24628). However, it is not known that this transgenic animal can use for the repair mechanism of breakage on a glomerulus as a renal dysfunction model animal which has a failure.

[0012] Homo sapiens Meg Singh is protein in which a code is carried out by DNA with the base sequence shown in array number:1. The presumed amino acid sequence is shown in array number:2. also using the animal which introduced DNA which carries out the code of the protein which has a function equivalent on not only Homo sapiens Meg Singh but Homo sapiens Meg Singh, and a functional target as a transgenic animal in this invention -- although -- it can do. As such protein, Meg Singh's homologue in other kinds

can be shown, for example. Structure of for example, Latt Meg Singh and mouse Meg Singh is clarified by this invention person at Meg Singh's homologue (WO 99/15652). An amino acid sequence is shown in array number:3, array number:4, and mouse Meg Singh's base sequence and a list, and an amino acid sequence is shown in Latt Meg Singh's base sequence, and a list array number:5 and array number:6.

[0013] Moreover, generally a polymorphism is shown that the gene of eukaryote is known for a Homo sapiens interferon gene in many cases. According to this polymorphism, even if it produces the permutation of the amino acid beyond one piece or it in an amino acid sequence, proteinic activity is usually maintained. Moreover, generally it is known for the alteration of the amino acid of one piece or some that proteinic activity will be maintained in many cases. Therefore, array number:2, array number:4, and an array number: The gene which carries out the code of the protein which consists of an amino acid sequence which changed artificially the amino acid sequence shown in either of 6 can be altogether used for this invention, as long as this protein brings a failure to the repair mechanism of breakage on a glomerulus.

[0014] The protein which has an equivalent function functionally is hereafter named generically Homo sapiens, Latt, or Meg Singh originating in a mouse, and it indicates as Meg Singh. In addition, even if it is the case where DNA which carries out the code of Meg Singh who originates in a mouse as Meg Singh is introduced into a mouse, DNA originating in the mouse introduced artificially is DNA of foreignness. However, as a renal dysfunction model animal which has a failure in the repair mechanism of breakage of this transgenic animal on a glomerulus, in order to screen a compound useful to the therapy agent in Homo sapiens, it is advantageous to use Homo sapiens Meg Singh's DNA. It is because possibility that the effect to Homo sapiens Meg Singh can be reflected more faithfully is expectable in the body of a transgenic animal.

[0015] Moreover, in consideration of the codon usage of the host who the codon to amino acid is well-known in itself, and the selection is also arbitrary, and is good, for example, uses, it can determine according to a conventional method [Grantham, R.et al.Nucleic Acids Res.9, and r43] (1981). Therefore, what changed DNA suitably is contained in DNA of this invention in consideration of the degeneracy of a codon. furthermore, a part of codon of these nucleic-acids array -- the site specific using the primer which consists of an synthetic oligonucleotide to which an alteration carries out the code of the desired alteration according to a conventional method -- a variation rate -- introducing method (sitespecific mutagenesis) [-- Mark, D.F.et al.Proc.Natl.Acad.Sci.U.S.A.81, 5662] (1984), etc. can be followed.

[0016] Furthermore, array number:1, array number:3, and an array number:

The DNA is contained in DNA by this invention as long as the protein can hybridize with DNA which includes the base sequence of a publication in either of 5, and a code is carried out [protein] by the DNA brings about the failure of the restoration function of the breakage in a glomerulus. It is thought that the array which can be hybridized in a specific array under stringent conditions has many in which a specific array has the protein which carries out a code, and similar activity. As conditions for washing, as "1xSSC, 0.1% SDS, 37-degree-C" extent and severer conditions, "0.5xSSC, 0.1% SDS, 42-degree-C" extent can be shown, and stringent conditions can usually show "0.1xSSC, 0.1%SDS, 55-degree-C" extent as still severer conditions. In addition, DNA which carries out the code of Meg Singh in this invention can also use the fragment, as long as the failure of the restoration function of the breakage in a glomerulus is brought to a transgenic animal.

[0017] DNA which carries out the code of Meg Singh used for production of a transgenic animal in this invention can be obtained by the well-known approach based on the base sequence indicated on these descriptions. For example, isolation of cDNA which carries out the code of Meg Singh is possible by screening as a probe DNA which consists of a base sequence which showed the cDNA library of a mesangial cell to array number:1, array number:3, or array number:5. Moreover, DNA which carries out the code of Meg Singh can be amplified by performing PCR by using this cDNA library as mold using the primer set up based on the base sequence shown in array number:1, array number:3, or array number:5. Cloning of the magnification product is carried out based on a well-known approach.

[0018] As for DNA which carries out the code of Meg Singh, it is advantageous to rearrange and to consider [which connected with the promotor who can be discovered in the cell of the animal which should introduce this gene] as a gene construct. The recombination gene construct of this invention can be built DNA which carries out the code of said Meg Singh to the vector in which cloning is possible using a suitable host, and by inserting a promotor and carrying out cloning to the upstream. As a promotor who can use for this invention, the fowl beta actin promotor who can guide the manifestation of a foreign gene by broad vertebrates, such as a mouse and Latt, can be shown.

[0019] Moreover, an enhancer is combinable in order to reinforce the manifestation of a foreign gene. For example, it is known that the enhancer originating in CMV will reinforce the manifestation of the foreign gene in mammalian. In construction of the recombination gene construct which consists of these genes, it can have an enhancer and a promotor and the vector which has arranged the multi-cloning site for foreign gene insertion on the lower stream of a river further can be used. The vector with such structure can be built by the approach as shown in an example based on

pCAGGS (Niwa H, Yamamura K and Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector.Gene 108,193-200.) etc. The rabbit beta globin terminator is arranged on the lower stream of a river of a multi-cloning site, and this vector contributes to improvement in the manifestation effectiveness of the inserted foreign gene.

[0020] With a suitable restriction enzyme, the recombination gene construct started from said vector is fully refined, and is used for production of a transgenic animal. A transgenic animal is produced by introducing said construct into the germinal cell containing an unfertilized egg, a fertilized egg, a sperm, and its progenitor cell etc. As a cell which introduces a construct, it is the phase of a single cell or an amphicytula, and the thing before 8 cell terms is usually used for the phase of the embryogenesis in generating of a nonhuman mammal, and a twist concrete target. as the introductory approach of a construct -- a calcium phosphate method, an electric pulse method, the RIPOFE cushion method, a condensation method, a microinjection method, and party Kurgan -- law, the DEAE-dextran method, etc. are well-known. Furthermore, a transgenic animal is also producible by uniting with an above-mentioned germinal cell the transformed cell obtained in this way.

[0021] The cell which introduces a construct can be a cell originating in all the nonhuman vertebrates that can produce a transgenic animal. Specifically, cells, such as a mouse, Latt, a hamster, a guinea pig, a rabbit, a goat, a sheep, Buta, a cow, a dog, or a cat, can be used. For example, in a mouse, the fertilized egg which can introduce a construct is recoverable by making the mouse of Metz which prescribed the ovulation inducing drug for the patient cross the mouse of a normal male. Generally in a mouse fertilized egg, a construct is introduced by the microinjection to male pronucleus. What is considered that the cell which introduced the construct succeeded in installation after culture of night extent in the outside of the body is transplanted to a surrogate mother's oviduct, and a transgenic chimera animal is born. Metz which was made to cross with the male which cut the spermatic duct, and was made into the pseudopregnancy condition is used for a surrogate mother.

[0022] The produced transgenic chimera animal is made to cross with an animal normal for birth of F1 animal after checking that the foreign gene (DNA which carries out the code of Meg Singh) is included in the genome by analyzing the gene of the somatic cell. At this time, an individual with desirable more many copy numbers is chosen. A multiple copy is included in a part with the genome same [DNA of the foreignness generally introduced as a construct] by the serial. Usually, it is because it leads to a lot of gene expression and a clearer manifestation mold can be expected, so that there are many these inclusion copy numbers. In a somatic cell genome, it can

check to a construct that the foreign gene (DNA which carries out the code of Meg Singh) is incorporated in the direction of the right by PCR using a specific primer. Moreover, the relative comparison of a copy number is possible by dot blotting methods.

[0023] What equips a somatic cell with a foreign gene (DNA which carries out the code of Meg Singh) in F1 animal born as a result of this mating is the transgenic animal which can tell a foreign gene (DNA which carries out the code of Meg Singh) to a reproductive cell with heterozygote (heterozygote). Therefore, what holds a foreign gene (DNA which carries out the code of Meg Singh) to a somatic cell is chosen from F1 animals, and if F2 animal which makes these parents can be made, the homozygote animal (homozygote animal) which holds a foreign gene (DNA which carries out the code of Meg Singh) by the gay will be obtained as F2 animal.

[0024] As long as DNA of Meg Singh of foreignness is discovered by the mesangial cell, even if it is which generation of these transgenic animals, it can use for the renal dysfunction model animal which has a failure in the repair mechanism of breakage on the glomerulus of this invention. For example, if Meg Singh of this foreignness is discovered by the mesangial cell even if it is the transgenic animal which holds Meg Singh's DNA by the hetero, it is useful as a renal dysfunction model animal which has a failure in the repair mechanism of breakage on a glomerulus.

[0025] In addition, in this invention, if DNA of Meg Singh of foreignness can be made to discover by the mesangial cell at least, it can consider as the renal dysfunction model animal of this invention. Therefore, it is not necessary to make a mesangial cell and a kidney unique target not necessarily discover DNA of Meg Singh of foreignness. For example, as shown in an example, even if it is the transgenic mouse which discovers HITOMEGUSHIN to generalized, it can consider as the renal dysfunction model animal which has a failure in the repair mechanism of breakage on a glomerulus.

[0026] That a transgenic animal has a failure in the repair mechanism of breakage on a glomerulus can do breakage on a glomerulus to the animal in the condition of not having abnormalities in a glomerulus, and it can check it by comparing with contrast. An animal with clear not having a failure in the repair mechanism of breakage is used for contrast. For example, the mouse of a wild type is desirable as contrast. The breakage on a glomerulus can be known by measuring the marker of a kidney function. For example, common kidney function markers, such as albumin in a serum creatinine value or urine, can be used. The measuring method of these kidney function markers is well-known. In addition, morphological change of a glomerulus organization can also be made into the index of breakage. For example, by the PAS stain of kidney tissue, the number of mesangial cells and the area of a mesangium

substrate can be observed, and extent of proliferative glomerulonephritis can be scored.

[0027] The animal which reinforced Meg Singh's manifestation in the glomerulus can be used for the repair mechanism of the breakage in a glomerulus as a model animal which has a failure. As for the animal which reinforced Meg Singh's manifestation in the mesangial cell, in this invention, it is desirable not to accept the abnormalities in a glomerulus in the condition of not doing breakage over a glomerulus. That is, the original function of a glomerulus is maintained and the animal by which the restoration function of breakage is barred specifically is desirable. By fulfilling such conditions, the condition of restoration of breakage is specifically observable. For example, in screening of the remedy which acts on a repair mechanism, the singularity of the failure over the restoration function of breakage influences the singularity of the screening. Therefore, by the screening approach using the model animal which reaches in addition to a repair mechanism, a failure may choose the compound which has the activity which acts in addition to a repair mechanism. Since the failure of the repair mechanism is carried out specifically, the renal dysfunction model animal of this invention can evaluate the activity over a repair mechanism specifically.

[0028] The following description can also define the renal dysfunction model animal of this invention.

(a) The renal dysfunction model animal of this invention in which the hemodialysis function and the gestalt-description of the (b) glomerulus which the Meg Singh gene expression in a glomerulus is reinforcing have such a normal description substantially can be made into the condition that the failure of the restoration of breakage was carried out easily, by doing breakage to a glomerulus.

[0029] In this invention, the hemodialysis function and the gestalt-description of a glomerulus say substantially maintaining the condition as an animal with these normal descriptions that normal is the same. When a kidney function marker is a normal value, it can know that a hemodialysis function is normal. As a kidney function marker, serum creatinine can be made into an index, for example. Moreover, it can check that the gestalt-description of a glomerulus is normal by not seeing the abnormality opinions on growth of hypertrophy of a glomerulus, substrate hyperplasia, or a mesangial cell etc.

[0030] Moreover, in this invention, the method of doing breakage on a glomerulus is arbitrary. Each method of doing well-known renal dysfunction can be used for this invention. It can consider as the approach of damaging a glomerulus, for example, the following approaches can be shown.

Administration of the drugs which do breakage to the administration glomerulus of an antigen which guides the antibody which recognizes the administration glomerular capillary basement membrane (GBM) antigen of the

antibody which recognizes a glomerular capillary basement membrane (GBM) antigen [0031] The approach of prescribing especially an anti-GBM antibody for the patient in these approaches can be said to be a desirable approach for the following reasons. That a glomerulus can be damaged promptly, that the effect which it has on a glomerulus is transient, that individual difference cannot appear easily in breakage on a glomerulus, [0032] An anti-GBM antibody can be obtained by carrying out immunity of the different animal from a model animal with the GBM antigen of a model animal. The method of obtaining an anti-GBM antibody is well-known (635 Hisada etal. and Angiotensin II plays a pathogenic role in immune-mediated renal injury in mice.J.Clin.Invest.103:627- 1999). The immune animal for obtaining an anti-GBM antibody is not limited. Furthermore, an anti-GBM antibody may be antiserum, and after considering as a purification antibody, it can also be prescribed for the patient. Or a monoclonal antibody can also be used as an anti-GBM antibody.

[0033] In order to replace with an anti-GBM antibody and to guide an anti-GBM antibody to a model animal by administration of a GBM antigen, a model animal is medicated with a GBM antigen with a suitable adjuvant. Or breakage can also be done to a glomerulus by administration of the compound which does a failure to a kidney function.

SUTOREPUTOZODOSHIN, habu venom, etc. can be shown as a compound which does breakage to a glomerulus.

[0034] In this invention, when using the Meg Singh transgenic animal for the animal which reinforced Meg Singh's manifestation in the mesangial cell, it is desirable to use the young individual of week-old before the abnormality opinion in a mesangial cell is found out. Specifically, the Meg Singh transgenic animal for 11-13 weeks is desirable as week-old, 10-15, 5-20 and an animal which reinforced Meg Singh's manifestation in the mesangial cell of this invention. [for example,] Morphologically or functionally, abnormalities are not looked at for the Meg Singh transgenic animal of this stage by the mesangial cell. However, the restoration function of the breakage in a glomerulus is falling remarkably. Therefore, if a certain breakage can be done to a glomerulus, restoration of the breakage will be remarkably overdue as compared with a wild type.

[0035] For example, as the renal dysfunction model mouse of this invention which was able to do breakage to the glomerulus by administration of an anti-GBM antibody was shown in the example, neither the function of a glomerulus nor the breakage on a gestalt will be restored after antibody administration also on the 28th. The abnormalities of the glomerulus of this stage were getting worse further compared with the result of the 7th day after antibody administration. On the other hand, with the wild type mouse which does not have a failure in a restoration function, abnormalities are not

accepted in the glomerulus on the 28th. It turns out that breakage extent of the glomerulus on the 7th is restored nearly thoroughly [breakage on a glomerulus] in between [7 day -28 day] with the wild type since a difference is not seen among both.

[0036] It is 35-40 weeks that the Meg Singh TRANS GENIC mouse presents abnormalities to a glomerulus by Meg Singh's compulsive manifestation. On the other hand, continuation of the abnormalities of the glomerulus shown in the example is the phenomenon observed at 15-19 weeks, having applied. Even if the Meg Singh transgenic animal is in the condition that the abnormalities of a glomerulus are not seen, it is proved by these knowledge that it has the failure in the repair mechanism of the breakage in a glomerulus.

[0037] Meg Singh is SERPIN. It has the structural description of the protease inhibitor belonging to a super family. It is predicted to be the cause of the failure of restoration to a protease required for restoration of the breakage in a glomerulus that Meg Singh works in inhibition. It has also supported this prediction that the failure of the mesangial cell in the Meg Singh transgenic animal is not seen at the stage when week-old is young. That is, by Meg Singh, as a result of carrying out the failure of the repair mechanism over a long period of time, breakage is accumulated and it results in renal dysfunction.

[0038] The renal dysfunction model animal obtained by this invention has a failure in the function which restores breakage on a glomerulus. Such a model animal is not known until now. Various renal dysfunction models are reported by laboratory animals, such as a mouse. However, doing breakage over a glomerulus was only taken into consideration in the well-known model. In such a model, even if it can inquire for preventing the cause of breakage, and it, research of a repair mechanism cannot be done.

[0039] The repair mechanism of the breakage in a glomerulus is the important structure for understanding human renal dysfunction. Now, the effective therapy approach for the nephritis which became chronic is not learned. In Homo sapiens, it is an important research technical problem to improve the function which restores breakage on a glomerulus. However, in laboratory animals, such as a mouse, breakage on a glomerulus will be restored in many cases. Therefore, in order to have reproduced the renal dysfunction which became chronic by the laboratory animal, breakage over a glomerulus needed to be done continuously. This means that the renal dysfunction in which Homo sapiens became chronic is unreproducible especially with the well-known renal dysfunction model obtained by the laboratory animal. One of the causes that the therapy approach of the renal dysfunction which became chronic is not developed has a repair mechanism in the symptoms by which the failure was carried out being artificially

unreproducible.

[0040] Since a repair mechanism can reproduce the condition that the failure was carried out, the model animal of this invention contributes to the research on restoration of breakage on a glomerulus. Specifically, the large-scale drugs screening for the remedy development which promotes a break through of the repair mechanism of the breakage in a glomerulus and restoration of breakage by the model animal of this invention is realizable.

[0041] The curative effect of the test compound to the failure of the repair mechanism of the breakage in a glomerulus can be evaluated using the model animal which has a failure in the repair mechanism of the breakage in the glomerulus of this invention. The assessment approach by this invention is enforced according to the following processes.

(1) The process which prepares the renal dysfunction model animal from which the restoration function of the breakage in a glomerulus to have the following description (a) and (b) was prevented, (a) -- the hemodialysis function and the gestalt-description of the (b) glomerulus which the Meg Singh gene expression in a glomerulus is reinforcing -- substantial -- normal (2) -- the process which does breakage to the glomerulus of said animal -- (3) The process which medicates said animal with a test compound in front of a process (2) and/or in the back, And the compound for a therapy for the failure of the repair mechanism of the breakage in a glomerulus can be screened using the model animal which has a failure in the repair mechanism of the breakage in the glomerulus of the process which evaluates extent of the recovery of damage in (4) glomeruli, and this invention. The screening approach of this invention can measure the activity which reinforces the restoration function of the failure in the glomerulus of a test compound by the assessment approach, and as compared with the contrast which does not prescribe a test compound for the patient, when said activity chooses a large compound, it can carry it out in the first half.

[0042] In the assessment approach of this invention, or the screening approach, extent of the recovery of damage of a glomerulus can evaluate a kidney function marker as an index. For example, the following kidney function markers are known. The approach for measuring these kidney function markers is also well-known.

the urea azoturia in the creatinine value blood in blood -- before and after prescribing the alpha one acid glycoprotein in beta2in albuminuria in inside hemoglobinuria-micro globulinuria, therefore a test compound for the patient, the effectiveness as a remedy of a test compound can be evaluated by comparing the observation result of these indexes. Or if an affiliated transgenic animal is used, the effectiveness between test compounds can also be compared by comparing the observation result of these indexes between animals.

[0043] As a test compound used for screening of this invention, nature or a synthetic compound, various organic compounds, nature or the compounded saccharide, protein, a peptide, the manifestation product of a gene library, a cell extract, or a biomass component can be mentioned, for example. In addition, the antisense nucleic acid which controls Meg Singh's manifestation, and the anti-MEGUSHIN antibody it is expected that Meg Singh's activity control is can also be made into a test compound. The renal dysfunction model animal of this invention is medicated with these test compounds in taking orally or parenterally.

[0044] Before a test compound does breakage to said glomerulus, a renal dysfunction model animal can be medicated with it behind. However, in order to find out the compound which acts more specifically to the restoration process of breakage, after doing breakage, it is desirable to prescribe a test compound for the patient. A more effective administration stage can also be clarified by changing the timing of administration of a test compound and comparing the operation over a restoration function.

[0045] The test compound chosen by the screening approach of this invention can be made into the active principle of the remedy constituent for a therapy for restoration of the breakage in a glomerulus after examining safety, stability, etc. further. The remedy constituent of this invention can be pharmaceutical-preparation-ized according to a well-known galenical pharmacy-manufacturing method, and can be prescribed for the patient. Moreover, the compound itself which is an active principle can also be directly prescribed for the patient. When pharmaceutical-preparation-izing, a medicine can be prescribed for the patient, combining suitably the medium or support generally used as drugs.

[0046] Moreover, if the code of this compound is carried out by DNA and it gets, this DNA will be included in the vector for gene therapies, and performing gene therapy will also be considered. Administration can be performed by approaches, such as intraarterial injection, an intravenous injection, the administration in a nasal cavity, administration in a bronchial tube, intramuscular administration, hypodermic administration, internal use, and direct administration to the affected part. A dose is changed according to conditions, such as a medication method, whenever healthy, a patient's weight, age, and, but if it is this contractor, it can choose a suitable dose suitably.

[0047] That is, for example in the renal dysfunction model animal of this invention, effective concentration is determined by comparing the enhancing effect of a breakage restoration function among various doses. And a dose to which the concentration of the administration compound in a mesangial cell reaches the effective concentration by each above administration roots is determined experientially. In a general administration gestalt, the dose per

weight of 1kg is determined as that from which an active principle is distributed over the whole body. If it is the compound considered that kidney translatability is high based on the analysis result of the pharmacokinetics and metabolism in a laboratory animal, a dose can be set up lower.
[0048] The remedy constituent of this invention is blended with a medium or support in consideration of the dose and administration gestalt which were determined. This contractor is usually performing blending an active principle so that a required dose can be attained. More generally 1micro [of usual / per weight of 1kg] g-10mg of doses of the remedy constituent by this invention can be set to 10micro g-1mg. Moreover, in the case of injections, about [of internal use] 1/100 can be made into the rule of thumb of a dose. A dose can be adjusted by performing a still more nearly special dosage form design. For example, in such pharmaceutical preparation, although it can also consider as gradual release-ized pharmaceutical preparation by holding to suitable support, since the remedy constituent of this invention can maintain high blood drug concentration, it can set up loadings low.
[0049]
[Example] [Example 1]: In order to create the construct for MEGUSHIN transgenic-mouse HITOMEGUSHIN transgenics, subcloning of Meg Singh's cDNA coding sequence was carried out to pBsCAG-2 (222 Kawarabashi T.et al., Accumlation of beta-amyloid fibriols in pancreas of transgenic mice.Neurobiol.Aging 17:215- 1996) in the sense direction. The structure of a construct was shown in drawing 1 . It is [a part of second intron, the third exon, and] perfect length HITOMEGUSHIN cDNA 3' - Subcloning was carried out into the rabbit beta globulin gene containing UTR. The location of the primer for PCR analysis was shown in the above-mentioned construct. It imported into the uterine tube of the mouse which carried out microinject of the Meg Singh recombination gene which cut pBsCAG-2 including Meg Singh DNA and was isolated to the fertilized pronucleus of B6C3F1 X C57BL / 6-N hybrid egg, and carried out pseudopregnancy according to the publication (Hogan B.et al., Manipulating the Mouse Embryo:A Laboratory Manual.Cold Spring Harbor NY:Cold Spring Harb or Laboratory.1986) independently.
[0050] The mouse genomic DNA extracted from the organization of a tail was used for detection of the recombination gene by the Southern blot analysis using the probe of the Meg Singh recombination gene. Moreover, a transgenic mouse is PCR using a primer specific to Meg Singh or pBsCAG-2 vector. It identified. The fragment of 250bp was amplified by the primer for the cytomegalovirus enhancer Pr 1, CMV-F1 (array number: 7), and CMV-R1 (array number: 8). The fragment of 400bp was amplified by a primer specific to the joining segment by the side of 5' between a vector and the inserted Meg Singh gene (Pr2), beta-g 1-3, and (array number: 9) hM 2-2 (array number: 10). The fragment of 563bp was amplified by a primer specific to the

joining segment by the side of 3' between a vector and the inserted Meg Singh gene (Pr3), hM 8-1 (array number: 11), and beta globin R (array number: 12).
CMV-F1:5'-GTC GAC ATT GAT TAT TGA CTA G-3' CMV-R1:5'-CCA TAA GGT CAT GTA CTG-3 ' beta-g 1-3:5'-CTT CTG GCG TGT GAC CGG CG-3' hM2-2:5'-ATC GAA TTC TGA GAT CAT AAT CCC TGT GGG ATG C-3' hM8-1:5'-TTA TTC AGT GGC AAA GTT TCT TGC CTT TGA-3' The individual from which a magnification product is acquired by all PCR by the primer of -3'3 pairs of beta globin R:5'-TCGAGG GAT CTT CAT AAG AGA AGA G It sorted out. Made obtained F zero-generation six individuals (male 3 individual, female 3 individual) cross with a normal individual (C57BL/6N Jcl), and obtained F1 generation, F1 of a hetero was made to cross with a normal individual further, and F2 was obtained.
[0051] [Example 2] anti-GBM antibody nephritis rabbit anti-GBM antiserum changed and prepared the well-known approach (635 Hisada et al. and Angiotensin II plays a pathogenic role in immune-mediated renal injury in mice.J.Clin.Invest.103:627- 1999). A GBM nephritis was guided to the 8-weeks old Meg Singh TRANS GENIC mouse (F3 or F4) and the wild type mouse of litter. Immunity of the mouse was carried out by medicating 0.025mg per weight of 1g, and the abdominal cavity with the rabbit IgG (Organon, Teknika, West Chester, PA) suspended in the Freund complete adjuvant. The anti-GBM antiserum of 100microL was injected intravenously from the caudal vein five days after immunity. In addition, it is checked that the anti-GBM antiserum of 100microL guides the glomerulonephritis accompanied by amplification of a mesangium field, and is thoroughly recovered the four months after by preliminary experiment. The mouse was slaughtered on three days (n= 5), the 7th (n= 5), and the 28th (n= 10) the anti-GBM antiserum processing to the Meg Singh TRANS GENIC mouse and a wild type mouse, and 6 hours after (N= 5). the kidney of a mouse -- a PAM stain (periodic acid-methenamine-silver stain) -- or PAS stain (periodic acid-Schiff stain) was carried out, and amplification of a mesangium substrate was evaluated according to the following scores 1-4.
1: Normal 3 : weakness/mild (glomerulus loop region; 1/3 or more [of glomerular tuft area])
4: Inside/moderate (glomerulus loop region; 2/3 or less [of glomerular tuft area])
5: Strength/severe (glomerulus loop region; 2/3 or more [of glomerular tuft area])
The glomerulus was scored when two pathologists observed the cross section of 20 or more glomeruli with a blind independently.
[0052] The deposition of the immune complex in a glomerulus was measured with the fluorescent antibody technique. That is, about a frozen section, they

are the fluorescein isothiocyanate (FITC) indicator goat anti-mice IgG, IgA, and IgM. Or it incubated with C3 antibody (Cappel). In order to compare the deposition of Mouse IgG, the following half-quantum scoring systems were used.
1: The glomerulus was scored when a deposition 3:25-50%4:50-75%5:>75%2 person pathologist observed the cross section of 20 or more glomeruli with a blind independently to normal 2:0-25% of glomerulus field.
[0053] Serum creatinine level was measured by the following approach. The creatinine which exists in a specimen is first decomposed in an operation of creatinine amidinohydrolase, a ZARUKOSHIN oxidase, and a catalase as the 1st reaction. Next, as the 2nd reaction, the creatinine in a specimen serves as a creatine in an operation of creatinine amide hydrase, and, subsequently produces Zarko Singh by creatine amidinohydrolase. It asked for creatinine concentration by carrying out oxidization condensation of N-ethyl-N(3-sulfopropyl)-3-methylaniline (TOPS) and the 4-aminoantipyrine for the hydrogen peroxide generated by the ZARUKOSHIN oxidase from Zarko Singh who arose under existence of a par oxidase, and measuring the absorbance of the quinone coloring matter to generate.
[0054] To the Meg overSingh manifestation mouse which gave stress, in order to check whether a glomerulopathy (glomerulopathy) is seen, an anti-GBM nephritis was guided to the transgenic mouse and the wild type mouse, and extent of amplification of a mesangium substrate was evaluated (drawing 2 and drawing 3). In the transgenic mouse, amplification of a mesangium substrate continued 28 days after. At this time, amplification of a mesangium substrate has improved with the wild type mouse. Are recording of the mouse IgG in the glomerulus on the 28th was same extent fundamentally among both as a result of half-quantum-analysis. There was no difference with time in the serum creatinine level of a transgenic mouse and a wild type mouse.

	トランスジェニック	野生型
7日目	0.11±0.01mg/dL	0.10±0.02mg/dL
28日目	0.09±0.03mg/dL	0.08±0.04mg/dL

[0055]
[Effect of the Invention] It is one of the causes which make the therapy of a disease difficult that the repair mechanism of a kidney organization by which the failure was carried out only not only in the failure of a kidney function in the patient who has abnormalities in a actual kidney function is inadequate. Many causes of a disease which cause renal dysfunction by current were clarified, and the research for removing the cause of a disease has also progressed. However, the model animal which has a failure in the repair

mechanism of a kidney function was not known, and retrieval of the drugs which promote research and restoration of a repair mechanism was not tried.

[0056] Research of the drugs which have the function which promotes the repair mechanism of a carrier beam kidney organization and restoration for breakage by the model animal of this invention is attained. By the model animal to which the failure of the repair mechanism of a kidney organization was carried out, the device in which restoration does not progress in a patient's kidney organization actually can be solved. Moreover, by the model animal of this invention, retrieval of the drugs which reinforce a restoration function also becomes possible. As long as the well-known model animal created by the failure of a kidney organization is used, retrieval of the drugs which have such a function cannot be performed.
[0057] By the model animal of this invention, a symptoms break through of the restoration malfunction of kidney tissue damage is attained. In much kidney disease except acute glomerulonephritis, it is thought that a failure is in the restoration function in a glomerulus, and the role which the model animal of this invention plays is large in a break through of the symptoms of these diseases, or retrieval of the therapy approach. By the model animal of this invention, the drugs which bring about enhancement of the restoration function of kidney tissue damage of such kidney disease can be screened.
[0058]
[Layout Table]
SEQUENCE-LISTING<110> MIYATA, Toshio
Japan-Science-and-Technology Corporation.<120> A renal-dysfunction-model animals<130> KRK-A0201<140><141><160> 15<170> PatentIn Ver. 2.0<210> 1<211> 1143<212> DNA<213> Homo sapiens<220> <221> CDS<222> (1).. (1140) <400> 1 atg gcc tcc ctt gct gcagca aat gca gag ttt tgc ttc aac ctgttc 48 Met Ala Ser Leu Ala Ala Ala Asn Ala Glu Phe Cys Phe Asn Leu Phe 1 5 10 15 aga gag atg gat gac aat caa gga aat gga aat gtg ttc ttt tcc tct 96 Arg Glu Met Asp Asp Asn Gln Gly Asn Gly Asn Val Phe PheSer Ser 20 25 30ctg agc ctc ttcgct gcc ctg gcc ctggtc cgc ttg ggc gct caa gat 144 Leu Ser Leu Phe Ala Ala Leu Ala Leu Val Arg Leu Gly Ala Gln Asp 35 40 45 gac tcc ctc tctcag att gat aag ttg ctt cat gtt aac act gcc tca 192 AspSer Leu Ser Gln Ile Asp Lys Leu Leu His Val Asn Thr Ala Ser 50 55 60 gga tat ggaaac tct tct aat agt cag tca ggg ctc cag tct caa ctg 240 Gly Tyr Gly Asn Ser Ser Asn Ser Gln Ser Gly Leu Gln Ser Gln Leu 65 70 75 80aaaaga gtt ttt tct gat ata aat gca tcc cac aag gat tat gat ctc 288 Lys Arg Val Phe Ser Asp Ile Asn Ala Ser His Lys Asp Tyr Asp Leu 85 90 95 agc att gtg aat ggg ctt ttt gct gaa aaa gtg tat ggcttt cat aag 336 Ser Ile Val Asn Gly Leu Phe Ala Glu Lys Val Tyr Gly Phe His Lys 100 105 110 gac tacatt gag tgt gcc gaa aaa tta tac gat gccaaa gtg gag cga 384 Asp Tyr Ile Glu

Cys Ala Glu Lys Leu Tyr Asp Ala Lys Val Glu Arg 115 120 125 gtt gac ttt acgaat cat tta gaa gac act aga cgt aat att aat aag 432 Val Asp Phe Thr Asn His Leu Glu Asp Thr Arg Arg Asn Ile Asn Lys 130 135 140 tgg gtt gaaaat gaa aca cat ggc aaa atc aag aac gtg att ggt gaa 480 TrpVal Glu-Asn-Glu-Thr-His Gly Lys Ile Lys Asn-Val-Ile-Gly-Glu145 150 155 160ggt ggc ata agc tca tct gct gta atg gtg ctg gtg aat gct gtg tac 528 Gly Gly Ile Ser Ser Ser Ala Val Met Val Leu Val Asn Ala Val Tyr 165 170 175 ttc aaa ggc aag tgg caa tca gcc ttc acc aag agc gaa acc ata aat 576 Phe Lys Gly Lys Trp Gln Ser Ala Phe Thr Lys Ser Glu Thr Ile Asn 180 185 190 tgc cat ttc aaa tct ccc aag tgc tct ggg aag gca gtc gcc atg atg 624 Cys His Phe Lys Ser Pro Lys Cys Ser Gly Lys Ala Val Ala Met Met 195 200 205cat cag gaa cggaag ttc aat ttg tct gtt att gag gac cca tca atg 672 His Gln Glu Arg Lys Phe Asn Leu Ser Val Ile Glu Asp Pro Ser Met 210 215 220 aagatt ctt gagctc aga tac aat ggt ggc ata aac atg tac gtt ctg 720 LysIle Leu Glu Leu Arg Tyr Asn Gly Gly Ile Asn Met Tyr Val Leu225 230 235 240 ctg cct gag aatgacctc tct gaa att gaa aac aaa ctg acc ttt cag 768 Leu Pro Glu Asn Asp Leu Ser Glu Ile Glu Asn Lys Leu Thr Phe Gln 245 250 255 aat cta atg gaa tgg acc aat cca agg cga atg acc tct aag tat gtt 816 AsnLeu Met Glu Trp Thr Asn Pro Arg Arg Met Thr Ser Lys Tyr Val 260 265 270 gag gta ttt ttt cct cag ttc aag ata gag aag aat tat gaa atg aaa 864 Glu Val Phe Phe Pro Gln Phe Lys Ile Glu Lys Asn Tyr Glu Met Lys 275 280 285caa tat ttg agagcc cta ggg ctg aaa gat atc ttt gat gaa tcc aaa 912 Gln Tyr Leu Arg Ala Leu Gly Leu Lys Asp Ile Phe Asp Glu Ser Lys 290 295 300 gcagat ctc tctggg att gct tcg ggg ggt cgt ctg tat ata tca agg 960 AlaAsp Leu Ser Gly Ile Ala Ser Gly Gly Arg Leu Tyr Ile Ser Arg305 310 315 320 atg atg cac aaatcttac ata gag gtc act gag gag ggc acc gag gct 1008 Met Met His Lys Ser Tyr Ile Glu Val Thr Glu Glu Gly Thr Glu Ala 325 330 335 act gct g cc aca gga agt aat att gta-gaa-aag-caa-ctc cct cag tcc 1056 Thr Ala Ala Thr Gly Ser Asn Ile Val Glu-Lys-Gln-Leu-Pro Gln Ser 340 345 350 acg-ctg-ttt-aga-gct gac cac cca ttc cta ttt gtt atc agg aag gat 1104 Thr Leu PheArg Ala Asp His Pro Phe Leu Phe Val Ile Arg Lys Asp 355 360 365 gac atc atc ttattc agt ggc aaa gtt tct tgc cct tga 1143 Asp Ile Ile Leu Phe Ser Gly Lys Val Ser Cys Pro 370 375 380 <210> 2<211> 380 <212> PRT <213> Homo sapiens<400> 2 Met Ala Ser Leu AlaAla Ala Asn Ala Glu Phe Cys Phe Asn Leu Phe 1 5 10 15 Arg Glu Met Asp Asp Asn GlnGly Asn Gly Asn Val Phe Phe Ser Ser 20 25 30 Leu Ser Leu Phe Ala Ala Leu Ala Leu Val Arg Leu Gly Ala Gln Asp 35 40 45 Asp Ser Leu Ser Gln Ile Asp Lys Leu Leu His Val Asn Thr Ala Ser 50 5560 Gly Tyr Gly Asn Ser Ser Asn Ser Gln Ser Gly Leu Gln Ser Gln Leu 65 70 75 80 Lys Arg Val PheSer Asp Ile Asn Ala Ser His Lys Asp Tyr Asp Leu 85 90 95 Ser Ile Val Asn Gly Leu Phe Ala Glu Lys Val Tyr Gly Phe His Lys 100 105 110 Asp TyrIle GluCys Ala Glu Lys Leu Tyr Asp Ala Lys Val Glu Arg 115 120 125 Val AspPhe Thr Asn His Leu Glu Asp Thr Arg Arg Asn Ile Asn Lys 130 135 140 Trp Val Glu Asn Glu Thr His Gly Lys IleLys

Asn Val Ile Gly Glu 145 150 155 160 Gly Gly Ile SerSer Ser Ala Val MetVal Leu Val Asn Ala Val Tyr 165 170 175 Phe LysGly Lys TrpGln Ser Ala Phe Thr Lys Ser Glu Thr Ile Asn 180 185 190 Cys His Phe Lys Ser ProL ys Cys Ser Gly Lys Ala-Val-Ala-Met-Met 195 200 205 His-Gln-Glu-Arg-Lys Phe Asn Leu Ser Val-Ile-Glu-Asp-Pro-Ser-Met 210 215 220 Lys-Ile-Leu-Glu Leu Arg Tyr Asn Gly Gly Ile Asn Met Tyr Val Leu 225 230235 240 Leu Pro Glu Asn Asp Leu Ser Glu Ile Glu Asn Lys Leu Thr Phe Gln 245 250255 Asn Leu MetGlu Trp Thr Asn Pro Arg Arg Met Thr Ser Lys Tyr Val 260 265 270 Glu Val Phe Phe Pro Gln Phe Lys Ile Glu Lys Asn Tyr Glu Met Lys 275 280 285 Gln Tyr Leu Arg Ala Leu Gly Leu Lys Asp Ile Phe Asp Glu Ser Lys 290 295300 AlaAsp Leu Ser Gly Ile Ala Ser Gly Gly Arg Leu Tyr Ile Ser Arg 305 310 315 320 Met Met His LysSer Tyr IleGluVal Thr Glu Glu Gly Thr Glu Ala 325 330335 ThrAla Ala ThrGly Ser Asn Ile Val Glu Lys Gln Leu Pro Gln Ser 340 345 350 Thr LeuPhe Arg Ala Asp HisPro Phe Leu Phe Val Ile Arg Lys Asp 355 360 365 Asp Ile Ile Leu Phe Ser Gly Lys Val Ser Cys Pro 370 375 380 <210> 3 <211> 1229<212> DNA <213> Rattus norvegicus<220><221> CDS <222> (8) .. (1147) <400> 3 tttcaaa atg gcc tcccttgct gca gca aatgcagaa ttt ggc ttcgac 49 Met Ala Ser Leu Ala Ala Ala Asn Ala Glu Phe Gly Phe Asp1 5 10 tta ttc aga gag atg gat agt agt caa gga aac gga aat gta ttc ttc 97 Leu Phe Arg Glu Met Asp Ser Ser Gln Gly Asn Gly Asn Val Phe Phe15 20 25 30tct tcc ctg agc atc ttc act gcc ctg agc cta atccgt ttg ggt gct 145 Ser Ser Leu Ser Ile Phe Thr Ala Leu Ser Leu Ile Arg Leu Gly Ala 35 40 45 cga ggt gac tgt nnn cgt cag att gac aag gcc ctg cac ttt atc tcc 193 Arg Gly Asp Cys Xaa Arg Gln Ile Asp-Lys-Ala-Leu-His Phe Ile Ser 50 55 60 cca-tca-aga-caa-ggg aat-tca-tcg-aac-agt cag cta gga ctg caa tat 241 Pro Ser Arg Gln Gly Asn Ser-Ser-Asn-Ser-Gln Leu Gly Leu Gln Tyr 65 70 75 caattg aaa aga gtt ctt gct gac ata aac tca tct cat aag gat nnn 289 Gln Leu Lys Arg Val Leu Ala Asp Ile Asn Ser Ser His Lys Asp Xaa 80 85 90 aaactc agc attgcc aat ggagtt ttt gca gag aaa gta ttt gat ttt 337 Lys Leu Ser Ile Ala Asn Gly Val Phe Ala Glu Lys Val Phe Asp Phe 95 100 105 110cat aag agc tat atg gag tgt gct gaa aac tta tac aat gct aaa gtg 385 His Lys Ser Tyr Met Glu Cys Ala Glu Asn Leu Tyr Asn Ala Lys Val 115 120 125 gaa aga gtt gat ttt aca aat gat ata caa gaa acc agattt aaa att 433 GluArg Val Asp Phe Thr Asn Asp Ile Gln Glu Thr Arg Phe Lys Ile 130 135 140 aat aaa tgg att gaaaat gaa aca cat ggc aaa atc aag aag gtg ttg 481 Asn Lys Trp Ile Glu Asn Glu Thr His Gly Lys Ile Lys Lys Val Leu 145 150 155 ggg gac agc agcctc agc tca tca gct gtc atg gtg cta gtg aat gct 529 Gly Asp Ser Ser Leu Ser Ser Ser Ala Val Met Val Leu Val Asn Ala 160 165 170 gtt tac ttcaaa ggc aag tgg aaa tcg gcc ttc acc aag agt gat acc 577 Val Tyr PheLys Gly Lys Trp Lys Ser Ala Phe Thr Lys Ser Asp Thr175 180 185 190ctc agt tgc cat ttc agg tct ccc agc ggt cct gga aaa gca gtt aat 625 Leu Ser Cys His Phe Arg Ser Pro Ser Gly Pro Gly Lys Ala Val Asn 195 200 205 atg atg cat caa gaa cgg agg ttc aat ttg tct acc

att cag gag cca 673 Met Met His Gln Glu Arg Arg Phe Asn Leu Ser Thr Ile Gln Glu Pro 210 215 220 cca atg cag att ctt gag cta caa tat cat ggt ggc ata agc atg tac 721 Pro Met Gln Ile Leu Glu Leu Gln Tyr His Gly Gly Ile Ser Met Tyr 225 230 235 atc atg ttg cccgag gat gac-cta-tcc-gaa-att gaa agc aag ctg agt 769 Ile Met Leu Pro Glu Asp Asp Leu-Ser-Glu-Ile-Glu Ser Lys Leu Ser 240 245 250 ttc-cag-aat-cta-atg gac tgg aca aat agc agg aag atg aaa tct cag 817 PheGln Asn Leu Met Asp Trp Thr Asn Ser Arg Lys Met Lys Ser Gln255 260 265 270tat gtg aat gtg ttt ctc ccc cag ttc aag ata gag aaa gat tat gaa 865 Tyr Val Asn Val Phe Leu Pro Gln Phe Lys Ile Glu Lys Asp Tyr Glu 275 280 285 atg agg agc cac ttg aaa tct gta ggc ttg gaa gac atc ttt gtt gag 913 Met Arg Ser His Leu Lys Ser Val Gly Leu Glu Asp Ile Phe Val Glu 290 295 300 tcc agg gct gat ctg tct gga att gcc tct gga ggt cgt ctc tat gta 961 Ser Arg Ala Asp Leu Ser Gly Ile Ala Ser Gly Gly Arg Leu Tyr Val 305 310 315tca aag cta atgcac aag tcc ctc ata gag gtc tca gaa gaa ggc acc 1009 Ser Lys Leu Met His Lys Ser Leu Ile Glu Val Ser Glu Glu Gly Thr 320 325 330 gaggca act gctgcc aca gaa agt aac atc gtt gaa aag cta ctt cct 1057 GluAla Thr Ala Ala Thr Glu Ser Asn Ile Val Glu Lys Leu Leu Pro335 340 345 350 gaa tcc acg gtgttcaga gct gac cgc ccc ttt ctg ttt gtc att agg 1105 Glu Ser Thr Val Phe Arg Ala Asp Arg Pro Phe Leu Phe Val Ile Arg 355 360 365 aag aat ggc atc atc tta ttt act ggc aaa gtc tcg tgt cct 1147 LysAsn Gly Ile Ile Leu Phe Thr Gly Lys Val Ser Cys Pro 370 375 380 tgaaattcta tttggttttccatacactaa caggcatgaa gaaacatcat aagtgaatag 1207 aattgtaatt ggaagtacat gg 1229 <210> 4 <211> 380<212> PRT <213> Rattus norvegicus <400> 4 Met Ala SerLeu Ala Ala AlaAsn Ala Glu Phe Gly Phe Asp Leu Phe 1 5 10 15 Arg Glu Met Asp Ser Ser Gln Gly AsnGly Asn Val Phe Phe Ser Ser 20 25 30 Leu Ser Ile Phe Thr Ala Leu Ser Leu Ile Arg Leu Gly Ala Arg Gly 35 40 45 AspCys Xaa Arg Gln Ile Asp Lys Ala Leu His Phe Ile Ser Pro Ser 50 55 60 Arg-Gln-Gly-Asn-Ser Ser Asn Ser Gln Leu-Gly-Leu-Gln-Tyr-Gln Leu 65 70 75 80 Lys Arg Val Leu Ala Asp Ile Asn Ser-Ser-His-Lys-Asp-Xaa-Lys-Leu 85 90 95 Ser Ile Ala Asn Gly Val Phe Ala Glu Lys Val Phe Asp Phe His Lys 100105 110 Ser TyrMet Glu Cys Ala Glu Asn Leu Tyr Asn Ala Lys Val Glu Arg 115 120125 ValAsp Phe Thr AsnAsp Ile Gln Glu Thr Arg Phe Lys Ile Asn Lys 130 135 140 Trp Ile Glu AsnGluThr His Gly Lys Ile Lys Lys Val Leu Gly Asp 145 150 155 160 Ser Ser Leu Ser Ser SerAla Val Met Val Leu Val Asn Ala Val Tyr 165 170 175 Phe Lys Gly Lys Trp Lys Ser Ala Phe Thr Lys Ser Asp Thr Leu Ser 180 185 190 Cys His Phe Arg Ser Pro Ser Gly Pro Gly Lys Ala Val Asn Met Met 195 200 205 His Gln Glu ArgArg Phe Asn Leu Ser Thr Ile Gln Glu Pro Pro Met 210 215 220 Gln Ile Leu Glu Leu Gln Tyr His Gly Gly Ile Ser Met Tyr Ile Met 225 230 235 240 Leu Pro Glu Asp Asp Leu Ser Glu Ile Glu Ser Lys Leu Ser Phe Gln 245 250 255 Asn Leu Met Asp TrpThr Asn SerArg Lys Met Lys Ser Gln Tyr Val 260 265 270 Asn ValPhe Leu Pro Gln Phe LysIle Glu Lys Asp Tyr

Glu Met Arg 275 280 285 Ser His Leu Lys Ser Val Gly Leu Glu Asp Ile Phe Val Glu Ser Arg 290 295 300 Ala Asp Leu Ser Gly Ile Ala Ser Gly Gly Arg Leu Tyr Val Ser Lys 305 310 315 320 Leu Met His Lys Ser Leu Ile Glu Val SerGlu Glu Gly Thr Glu Ala 325 330 335 Thr Ala AlaThrGlu Ser AsnIle Val Glu Lys Leu Leu Pro Glu Ser 340 345 350 Thr Val Phe Arg AlaAsp Arg Pro Phe Leu Phe Val Ile Arg Lys Asn 355 360 365 Gly-Ile-Ile-Leu-Phe Thr Gly Lys Val Ser-Cys-Pro 370 375 380 <210> 5<211> 1386<212> DNA<213> Mus musculus<220><221> CDS <222> (1) .. (1143) <<220> 221> misc_feature <222> (1354) .. (1354) <223> n represents an unspecified base. <400> 5 atg gcc tcc ctt gct gca gca aat gca gaa ttt ggc ttc gac tta ttc 48 MetAla Ser Leu Ala Ala Ala Asn Ala Glu Phe Gly Phe Asp Leu Phe 1 5 10 15 aga gag atg gat agt agc caa gga aat gga aat gta ttc ttc tct tcc 96 Arg Glu Met Asp Ser Ser Gln Gly Asn Gly Asn Val Phe Phe Ser Ser 20 25 30 ctg agc atc ttc act gcc ctg acc cta atc cgt ctg ggt gct cga ggt 144 Leu Ser Ile Phe Thr Ala Leu Thr Leu IleArg LeuGly Ala Arg Gly 35 40 45 gac tgt gca cgt cag att gac aaggca ctg cac ttt aac ata cca tca 192 Asp Cys Ala Arg Gln Ile Asp Lys Ala Leu HisPhe Asn Ile Pro Ser 50 55 60 aga caa gga aac tca tct aat aat cagcca gga ctt cag tatcaa ttg 240 Arg Gln Gly Asn Ser Ser Asn Asn Gln Pro Gly Leu Gln Tyr Gln Leu 65 70 75 80 aaaaga gtt ctt gct gac ata aac tca tct cat aag gattat gaa ctc 288 Lys Arg Val Leu Ala Asp Ile Asn Ser Ser His LysAsp Tyr Glu Leu 85 90 95 agc att gcc act gga gtt ttt gca gaa aaa gtc tatgac ttt cat aag 336 Ser Ile Ala Thr Gly Val Phe Ala Glu Lys Val TyrAsp Phe His Lys 100 105 110 aac tacatt gag tgt gct gaa aac tta tac aat gct aaa gtg gaa aga 384 Asn Tyr Ile Glu Cys Ala Glu AsnLeu Tyr Asn Ala Lys Val Glu Arg 115 120125 gttgac ttc aca aat gat gta caa gat accaga ttt aaa att aat aaa 432 Val Asp Phe Thr Asn Asp Val Gln Asp Thr Arg PheLys Ile Asn Lys 130 135 140 tgg-att-gaa-aat-gag aca cat gga aag atc-aag-aag-gtg-ttg-ggc-gac 480Trp Ile Glu Asn Glu-Thr-His-Gly-Lys Ile Lys Lys Val Leu-Gly-Asp 145 150 155160 agc agc ctc agc tcg tcg gct gtc atg gtg ctg gtg aac gct gtt tac 528 Ser Ser Leu Ser Ser Ser Ala Val Met Val Leu Val Asn Ala Val Tyr 165 170 175 ttcaaa ggc aaa tgg aaa tcg gcc ttc acc aag act gat acc ctc agt 576 Phe Lys Gly Lys Trp Lys Ser Ala Phe Thr Lys Thr Asp Thr Leu Ser 180 185 190 tgc cgt ttt agg tct ccc acg tgt cct gga aaa gta gtt aat atg atg 624 Cys Arg Phe Arg Ser Pro Thr Cys Pro Gly Lys Val Val Asn Met Met 195 200 205 catcaa gaa cgg cgg ttc aat ttg tct acc att cag cag cca cca atg 672 His Gln Glu Arg Arg Phe Asn Leu Ser Thr Ile Gln Gln Pro Pro Met 210 215 220 caggtt ctt gag ctc caa tat cat ggt ggc ata agc atg tac att atg 720 Gln Val Leu Glu Leu Gln Tyr His Gly Gly Ile Ser Met Tyr Ile Met 225 230 235 240 ctg cct gag gat ggc cta tgt gaa att gaa agc aag ctg agt ttc cag 768 Leu Pro Glu Asp Gly Leu Cys Glu Ile Glu Ser Lys Leu Ser Phe Gln 245 250 255 aatctg atg gac tgg acc aat agg agg aaa atg aaa tct cag tat gtg 816 Asn Leu Met Asp Trp Thr Asn Arg Arg Lys Met Lys Ser Gln Tyr Val 260 265 270 aac gtg ttt ctc ccc

cag ttc aag ata gag aag aat tat gaa atg acg 864 Asn Val Phe Leu Pro Gln Phe Lys Ile Glu Lys Asn Tyr Glu Met Thr 275 280 285 caccac ttg aaa tcc tta ggc ttg aaa gat atc ttt gat gag tcc agt 912 His His Leu Lys Ser Leu Gly Leu Lys Asp Ile Phe Asp Glu Ser Ser 290 295 300 gcagat ctc tct gga att gcc tct gga ggt cgt ctc tac gta tca aag 960 Ala Asp Leu Ser Gly Ile Ala Ser Gly Gly Arg Leu Tyr Val Ser Lys 305 310 315 320 cta-atg-cac-aag-tca ttc ata gag gtc tca-gag-gag-ggc-act-gaa-gcc 1008Leu Met His Lys Ser Phe Ile Glu Val-Ser-Glu-Glu-Gly-Thr-Glu-Ala 325 330 335 act gct gcc aca gaa aat aac att gtt gaa aag cag ctt cct gag tcc 1056 Thr Ala Ala Thr Glu Asn Asn Ile Val Glu Lys Gln Leu Pro Glu Ser 340 345 350 acagtg ttc aga gcc gac cgc ccc ttt ctg ttt gtc atc aag aag aat 1104 Thr Val Phe Arg Ala Asp Arg Pro Phe Leu Phe Val Ile Lys Lys Asn 355 360 365 gacatc atc tta ttt act ggc aaa gtc tct tgt cct tga aattcgattt 1153 Asp Ile Ile Leu Phe Thr Gly Lys Val Ser Cys Pro 370 375 380 ggtttcctat acagtaacag gcatcaagaa gcatcataagtgaatagaat tgtaattgga 1213 aatacatggg tttgctttga tttgtttcag tgcattgatg gtcagcaaat gatactggtg 1273acttaactct tttcgatacc tggttgatag tctaaatgcc tgctctcatc atttctactc1333catttacttc cctttgtcta ntactattga cccatctggg gtttgatcca ctc 1386 <210> 6 <211> 380 <212> PRT <213> Mus musculus<220><221> misc_feature <222> (1354) .. (1354) <223> nrepresents an unspecified base. <400> 6 Met Ala SerLeu Ala Ala Ala Asn Ala Glu Phe Gly Phe Asp LeuPhe 1 5 10 15 Arg Glu Met Asp Ser Ser Gln Gly Asn Gly Asn Val Phe Phe Ser Ser 20 25 30 Leu Ser Ile Phe Thr Ala Leu Thr Leu Ile Arg Leu Gly Ala Arg Gly 35 40 45 Asp Cys Ala Arg Gln Ile Asp Lys Ala Leu His Phe Asn Ile Pro Ser 50 55 60 Arg Gln Gly Asn Ser Ser Asn Asn Gln Pro Gly Leu Gln Tyr Gln Leu 65 70 75 80 Lys Arg Val Leu Ala Asp Ile Asn Ser Ser His Lys Asp Tyr Glu Leu 85 90 95 Ser Ile Ala Thr Gly Val Phe Ala Glu Lys Val Tyr Asp Phe His Lys 100 105 110 Asn Tyr Ile GluCys Ala Glu Asn Leu Tyr Asn Ala Lys Val Glu Arg 115 120 125 Val Asp Phe Thr Asn Asp Val Gln Asp Thr Arg Phe Lys Ile Asn Lys 130 135 140 Trp Ile Glu Asn Glu Thr His Gly Lys Ile Lys Lys Val Leu Gly Asp 145 150 155 160 Ser Ser Leu Ser Ser Ser Ala Val Met-Val-Leu-Val-Asn-Ala-Val-Tyr 165 170 175 Phe Lys Gly Lys Trp Lys Ser Ala Phe Thr-Lys-Thr-Asp Thr Leu Ser 180 185 190 Cys Arg Phe Arg Ser Pro Thr Cys Pro Gly Lys Val Val Asn Met Met 195 200 205 His Gln Glu Arg Arg Phe Asn Leu Ser Thr Ile Gln Gln Pro Pro Met 210 215 220 Gln ValLeu Glu Leu Gln Tyr His Gly Gly Ile Ser Met Tyr Ile Met 225 230 235 240 Leu Pro Glu Asp Gly Leu Cys Glu Ile Glu Ser Lys Leu Ser Phe Gln 245 250 255 Asn Leu Met Asp Trp Thr Asn Arg Arg Lys Met Lys Ser Gln Tyr Val 260 265 270 Asn Val Phe Leu Pro Gln Phe Lys Ile Glu Lys Asn Tyr Glu Met Thr 275 280 285 His His Leu Lys Ser Leu Gly Leu Lys Asp Ile Phe Asp Glu Ser Ser 290 295 300 Ala Asp Leu Ser Gly Ile Ala Ser Gly Gly Arg Leu Tyr Val Ser Lys 305 310 315 320 Leu Met His Lys Ser Phe Ile Glu Val Ser Glu Glu Gly Thr Glu Ala 325 330 335 Thr Ala Ala Thr Glu Asn Asn Ile Val Glu Lys Gln

Leu Pro Glu Ser 340 345 350 Thr Val Phe Arg Ala Asp Arg Pro Phe Leu Phe Val Ile Lys Lys Asn 355 360 365 Asp Ile Ile Leu Phe Thr Gly Lys Val Ser Cys Pro 370 375 380 <210> 7 <211> 22<212> DNA<213> Artificial Sequence<220><223> Description of Artificial Sequence:an artificially synthesized primer sequence <400> 7 gtcgacattg attattgact ag 22 <210> 8<211> 18<212> DNA <213> Artificial Sequence<220><223> Description of Artificial Sequence:an artificially synthesized primer sequence <400> 8ccataaggtc atgtactg 18 <210> 9 <211> 20<212> DNA <213> Artificial Sequence<220><223> Description of Artificial Sequence:an artificially synthesized primer sequence <400> 9 cttctggcgt-gtgaccggcg 20 <210> 10<211> 34<212> DNA<213> Artificial-Sequence<220> <223> Description-of-Artificial-Sequence:an-artificially synthesized primer sequence<400> 10atcgaattct gagatcataa tccctgtggg atgc 34 <210> 11 <211> 30<212> DNA <213> Artificial Sequence<220><223> Description of Artificial Sequence:an artificially synthesized primer sequence <400> 11ttattcagtggcaaagtttc ttgcccttga 30 <210> 12 <211> 25<212> DNA <213> Artificial Sequence<220><223> Description of Artificial Sequence:an artificially synthesized primer sequence <400> 12 tcgagggatc ttcataagag aagag 25

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing showing the structure of the construct for installation of a HITOMEGUSHIN gene.
[Drawing 2] The microphotography of a glomerulus [in / after processing of the wild type mouse (above) which guided an anti-GBM nephritis, and the Meg Singh TRANS GENIC mouse (below) / the 7th (left) day and the 28th (right) day]. (PAS stain, 200 times)
[Drawing 3] The graph which shows the result of the amplification half quantitive analysis of the mesangium substrate in an anti-GBM nephritis mouse. An axis of ordinate shows a score among drawing, and, as for O of a wild type mouse, in an axis of abscissa, the days after anti-GBM antiserum processing and ** show the result of a transgenic mouse.

[Translation done.]